THE EFFECT OF DIFFERENT MELANOSIS-INHIBITING BLENDS ON THE QUALITY OF FROZEN DEEP-WATER ROSE SHRIMP (*Parapenaeus longirostris*)

Oscar Martínez-Alvarez *, María Elvira López-Caballero, Pilar Montero and María del Carmen Gómez-Guillén

Institute of Food Science, Technology and Nutrition, ICTAN-CSIC, C/José Antonio Novais 10, 28040 Madrid, Spain**

 Corresponding author. E-mail address: oscar.martinez@ictan.csic.es

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ABSTRACT

The influence of different melanosis-inhibiting formulations on sensory parameters, texture, water holding capacity and microbiological properties of frozen deep-water rose shrimp (*Parapenaeus longirostris*) was evaluated throughout 6 months of storage at -18 ºC. Eight formulations were tested: three of them containing 4-hexylresorcinol (4-HR, 0.05, 0.1 and 0.25%, w/v) in combination with organic acids (citric, ascorbic and acetic) and chelating agents (ethylenediaminetetraacetic acid and di-sodium di-hydrogen pyrophosphate); four commercial formulae based on sulphites (alone or accompanied by gluconic acid, chitosan or glucose), and a commercial blend based on 4-HR and NaCl. Non-treated shrimp were used as control. 4-HR-based treatments, as well as the joint use of sulphites and D-gluconic acid, were very effective in preventing melanosis during storage without affecting sensory parameters. All formulations assayed did not alter the water holding capacity of muscle protein during frozen storage. In general terms, shrimp hardness increased after a melanosis-inhibiting blend treatment and remained more or less constant during storage. From the microbiological point of view, experimental formulations with 4-HR reduced the total microbiota after treatment, especially at 0.1% (w/w). After 6 months of frozen storage, for most microbial groups studied counts were lower than the limit of detection, except in shrimp treated with the commercial 4-HR-based formulation, where counts were close to 3 log cfu/g. The melanosis-inhibiting blends assayed were useful to preserve sensory, functional and microbial quality in shrimp after six months of frozen storage.

Keywords: Deep-water rose shrimp, 4-hexylresorcinol, freezing, crustaceans, melanosis, polyphenol oxidase

Abbreviations: 4-HR, 4-hexylresorcinol; NA, no additives; CS, control sulfite; GLN, gluconic acid; CH, chitosan; GLU, glucose.

1. Introduction

Deep-water rose shrimp (*P. longirostris*) is a highly appreciated crustacean captured in the Atlantic Ocean, mainly in the Gulf of Cadiz, in Morocco and in some areas of the Mediterranean Sea. Due mainly to market price and limited habitat, deep-water rose shrimp is not the most consumed species worldwide, yet it is the most sold crustacean in retail establishments in Andalusia (Spain). In Spain the market price of fresh deep-water rose shrimp has increased by a 25-50% in the past five years, reaching an average range of 15-50 euro/kilo in 2018, depending on the size. This shrimp is also commercialized cooked and/or frozen.

Deep-water rose shrimp are usually captured by small fishing boats which are rarely equipped with freezing chambers. Thus, fishermen must preserve captures refrigerated or stored in crushed ice boxes until reaching port. When deep-water rose shrimp are frozen inland, the delay in freezing can irreversibly affect quality, as melanosis develops very quickly in this crustacean species. Moreover, deep-water rose shrimp inhabit in cold water and deteriorate faster than warm-water species during ice storage, as documented by Goncalves, López-Caballero, & Nunes (2003). The optimum way to maintain and commercialize this species is at temperature approaching that of melting ice (close to 0°C) according to Regulation (EC) No 853/2004; still, at times they require to be frozen for long distance transport, as it is the only way to avoid spoilage.

Melanosis is considered a limiting factor for crustacean preservation. This alteration originates by the action of polyphenol oxidase (PPO) and in some species, as deep-water rose shrimp, also by the action of activated hemocyanin (Adachi, Hirata, Nagai, & Sakaguchi, 2001; Martínez-Alvarez, Gómez-Guillén, & Montero, 2008). PPO and activated hemocyanin oxidize phenolic compounds to quinones that spontaneously polymerize forming very high molecular weight pigments. Such pigments are not considered to be harmful to health; however, they devalue the commercial quality of the product. Melanosis-inhibiting blends, mainly those based on sulphite derivatives, are usually added after capture in order to avoid the appearance of black spots beneath the cuticle. Nevertheless, there is certain controversy related to the doses recommended by

manufacturers and the legislated residue limit in the edible portion of the crustacean, which often results in a high percentage of seizures. This fact, together with the occasional allergies and asthmatic episodes in certain population groups associated with the consumption of treated seafood (Vally & Misso, 2012), makes the search for new food-grade melanosis-inhibitors relevant. In this regard, 4-HR is viewed as an interesting alternative to sulphites in crustacean treatment. This compound is deemed GRAS (Generally Recognized as Safe) in the United States, where its commercial use for food purposes is allowed. In the European Union, the use of 4-HR as an antioxidant has been approved in fresh, frozen or deep-frozen crustacean meat, but maximum residue levels in the edible portion of the crustacean cannot exceed 2 mg/kg. Some studies support the effectiveness of 4-HR preventing melanosis in fresh crustaceans, including deep-water rose shrimp (López-Caballero, Martínez-Álvarez, Gómez-Guillén, & Montero, 2006b; López-Caballero, Martínez-Álvarez, Gómez-Guillén, & Montero, 2019). The use of polyphenols to avoid the appearance of black spots is another possibility that has received some attention among researchers (Nirmal, Benjakul, Ahmad, Arfat, & Panichayupakaranant, 2015; Sae-leaw & Benjakul, 2019). Chitosan dissolved in an acid medium has also been reported to be useful as a melanosis inhibitor (Simpson, Gagné, Ashie, & Noroozi, 1997), as well as the enzymatic glucose oxidase/catalase solution (Dondero, Egaña, Tarky, Cifuentes, & Torres, 1993).

Nearly all studies published on crustacean melanosis have been developed in refrigeration, where the appearance of blackening may occur within a few hours after capture. As a consequence, sometimes melanosis-inhibiting blends are added in excess. However, the distribution and consumption of frozen crustaceans constitutes a much larger market volume than that of fresh seafood and therefore, the negative impact of melanosis may have great economic significance. There is scarce information available on PPO activity in frozen crustaceans and its implication in melanosis, and there is even less information about the effect of melanosis-inhibiting formulations applied before freezing.

During frozen storage, protein denaturation frequently occurs as a consequence of the formation of ice crystals, dehydration and solute concentration in the tissues. This negatively affects the functional properties of myofibrillar proteins, characterized mainly by loss of water retention capacity and variations in muscle texture. Some physicochemical and functional properties of proteins during frozen preservation have been studied in different shrimp species (Jiang, Hwang, Chen, & Moody, 1991;

Srinivasan, Xiong, & Blanchard, 1997); nevertheless, the effect on deep-water rose shrimp is not known.

The aim of this work was to investigate the influence of different melanosis-inhibiting formulations on sensory parameters of deep-water rose shrimp (*P. longirostris*), frozen after capture and preserved at -18ºC for 6 months. A second objective was to determine the effect of those formulations on texture, water holding capacity and microbiological properties of frozen shrimp.

2. Materials and Methods

2.1. Chemicals

The commercial sulphite-based product (Freskor, maximum content of SO_2 = 60%) was from Hasenosa S.A. (Vigo, Spain). Chitosan was from Guinama S.L. (Valencia, Spain). The commercial 4-HR-based product (EverFresh®) was from Opta Food ingredients, Inc (Beldford, MA, USA). D-gluconic acid, 4-HR, reagent grade citric acid, ascorbic acid, proline and catechol were from Sigma Chemicals (St. Louis, MO, USA). Ethylene diamine tetra-acetic acid (EDTA), acetic acid, disodium dihydrogen pyrophosphate (PPi, 1.5 %, w/v), sodium hydroxide, perchloric acid and NaCl were from Panreac Chemicals Co. (Barcelona, Spain).

2.2. Sample preparation

Deep-water rose shrimp were caught off the South coast of Spain (Cádiz) by trawl in November. On board, they were separated from the by-catch and washed with seawater. The bulk sample was divided into 9 treatment groups of 2 kg each (Table 1). One of these groups was immersed in seawater and covered with crushed ice for one hour, and was considered the control group (lot NA). Freskor at 4% (w/w) was applied by immersion alone (lot CS), or accompanied by either 0.15 N gluconic acid (lot CS-GLN), 0.3% chitosan (CS-CHIT, w/w, previously dissolved in 0.05 N acetic acid), or 6 % glucose (CS-GLU, w/w). These melanosis-inhibiting blends were dissolved in seawater, and afterwards the shrimp were introduced into the blend and covered with crushed ice for 1 hour. The ratio shrimp:seawater:ice used was 1:2:1. EverFresh® (lot CR) was also applied by immersion (0.8%, w/w) for 5 minutes. Once the treatment was finished, the shrimp were removed, placed in perforated polystyrene boxes of 2 kg capacity and covered with ice. Additionally, 3 other lots were sprayed with different melanosis-inhibiting blends, including citric acid (0.5%, w/v), ascorbic acid (0.5%, w/v), acetic acid (0.05 N), EDTA (450 ppm, w/v), PPi (1.5 %, w/v) and different

concentrations (w/w) of 4-HR (0.25%, R-0.25; 0.1%, R-0.1; 0.05%, R-0.05). The 4-HR-based mixtures were previously diluted in approximately 1 litre of seawater. After spraying, shrimp were placed in perforated polystyrene boxes of 2 kg capacity and covered with crushed ice. Upon arrival of the trawler at port (about 24 h after capture), shrimp were deep frozen, stored at -18 ºC for 48 hours and transported inside an isothermal truck to the ICTAN (Spain), where they were stored at -18 ºC for 6 months. The batches were defrosted at 3ºC for further analyses.

2.3. Proximate analysis

Proximate analyses of shrimp after capture were performed according to the procedures of the Association of Official Analytical Chemists for moisture (method 950.46), ash (method 920.153) and total protein content (method 928.08), (A.O.A.C., 2002). Crude fat was determined as described by (Bligh & Dyer, 1959). Proximate analysis results of fresh shrimp were as follows: crude protein 21.04 ± 0.5%; moisture 76.99 ± 0.1%, total fat 0.40 ± 0.1% and ash 1.57 ± 0.10%.

2.4. Water holding capacity (WHC)

WHC was determined as described by Montero, Gómez-Guillén, & Borderías (1996). Two grams of homogenised muscle were cut, weighed, and placed in a centrifuge tube with three pipette filters (Gilson Pipetman, France) as absorbents. Samples were centrifuged for 10 min at 6000 x G in a Sorvall RT60008 centrifuge (DuPont Co., Delaware, USA) at room temperature. Filters were then weighed to calculate the amount of water retained by muscle protein. The results were the average of 3 measurements, expressed as percentage of water retained.

2.5. Shear strength

Shear strength was determined on two peeled tails by using an Instron Universal Testing Machine (Model 4464, Instron Engineering, Canton, Mass., USA) with a computer-controlled TA-XT2 Texture Analyser (Texture Technologies Corp., Scarsdale, NY). Peeled shrimp were previously thawed at low temperature and equilibrated to room temperature before analysis. They were placed in the shear cell perpendicular to the shear blades. The maximum force (N) and the area below the force curve (J) were obtained using a cell load of 5 kN applied at a crosshead speed of 100 mm/min. The results (average of 5 determinations) were expressed as Newton per gram of muscle at the point of maximum load before sample breaking.

2.6. pH

Approximately 5-10 grams of muscle were homogenized with double the amount (g/ml) of distilled water. After five minutes at room temperature, pH was determined with a pHm93 pH-meter and a combined pH electrode (Radiometer, Copenhagen, Denmark). The experiments were repeated at least in triplicate.

2.7. Microbiological assays

Ten-fifteen grams of muscle were firstly placed in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 ml of buffered 0.1 % peptone water (Oxoid, Basingstoke, UK) in a vertical laminar flow cabinet (mod. AV 30/70 Telstar, Madrid, Spain). After 1 min in a Stomacher blender (model Colworth 400, Seward, London, UK), appropriate dilutions were prepared for the following microorganism determinations: (i) total bacterial counts (TBC) on spread plates of Iron Agar 1% NaCl incubated at 15 ºC for 3 days; (ii) H_2S-producing organisms, as black colonies, on spread plates of Iron Agar 1% NaCl incubated at 15 ºC for 3 days; (iii) luminescent bacteria on spread plates of Iron Agar 1% NaCl incubated at 15 ºC for 5 days; (iv) *Pseudomonas* on spread plates of Pseudomonas Agar Base (Oxoid) with added CFC (Cetrimide, Fucidine, Cephaloridine) supplement for *Pseudomonas* spp. (Oxoid) incubated at 25 ºC for 48 h; (v) *Enterobacteriaceae* on double-layered plates of Violet Red Bile Glucose agar (VRBG, Oxoid) incubated at 30 °C for 48 h [after adding 5 ml of Tryptone Soy Agar (Merck, Darmstadt, Germany) and incubating at room temperature for 1 h]; (vi) lactic acid bacteria on double-layered plates of MRS Agar (Oxoid) incubated at 30 °C for 72 h. All microbiological counts were expressed as the log of the colony-forming units per gram of sample. All analyses were performed in triplicate.

2.8. Total volatile basic nitrogen (TVB-N)

TVB-N determinations were carried out in triplicate over the storage period using the method of Antonacopoulos & Vyncke (1989). Ten g ± 0.1 g of the ground sample were weighed in a suitable container and homogenized for two minutes with 100 ml of 6 % perchloric acid solution. After filtered, the extract was alkalinized with 20 % sodium hydroxide solution and submitted to steam distillation. The volatile base components were absorbed by an acid receiver and determined by titration of the absorbed bases. All analyses were performed at least in triplicate. The results were expressed as mg of nitrogen/100 g of sample.

2.9. Dimethylamine (DMA)

The amount of DMA in the samples was determined by gas chromatography as described by Torrejón, Del Mazo, Tejada, & Careche (1999). The gas chromatograph

(Perkin Elmer 8500, Perkin Elmer, Beaconsfield, UK) was equipped with a glass column (1.75 m, 2mm i.d.) packed with 25 cm untreated 80-100 mesh Chromosorb 103 and 150 cm of 4% Carbowax 20M+0.8% KOH on Carbopack B and a flame ionization detector (FID). The results were the average of 6 measurements and were expressed as mg per g of sample.

2.10. Colour

Lightness (+L*), yellowness (+b*), and redness (+a*) of dried and well-pulverized cephalothorax carapaces were measured with a Hunterlab colorimeter (Hunter Associates Laboratory, Inc., Reston, Virginia, USA), using a CIELab scale (Young & Whittle, 1985). Whiteness (W) was also calculated, as described by (Park, 1994). The results were the average of 6 measurements.

2.11. Diphenoloxidase activity determination

Crude enzyme was obtained from head carapaces according to Martínez-Alvarez, et al., (2008). Enzyme activity was measured using the proline-catechol spectrophotometric assay (Rzepecki & Waite, 1989) at saturating conditions, as described by Wang, Taylor, & Yan (1992). The results were expressed as nmol of proline-quinone adduct formed per minute. Analyses were performed in triplicate.

2.12. Sensory evaluation

A group of trained panellists routinely evaluated odour and shrimp appearance throughout frozen storage (10 individuals per treatment per evaluation). Melanosis was scored according to Martínez-Alvarez, López-Caballero, Montero, & Gómez-Guillén (2007) on a scale from 1 to 4, where 1= complete absence of black spots; 2= a few small spots on the carapace; 3= considerable spotting on the carapace; 4= substantial spotting over the entire shrimp. Odour was scored on a scale from 0 to 3, where 0= characteristic; 1= neutral; 2= a slight odour to ammonia or rancidity; 3= unpleasant (intense odour to ammonia or rancidity).

2.13. Statistical analyses

The significance of differences among mean values was evaluated using two-way ANOVA. Tukey's HSD procedure was used as post-hoc test, with a level of significance of $P \leq 0.05$. Statistical processing was performed by using the IBM SPSS version 25 computer program (Chicago, Illinois, USA). The level of significance setting was $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Water holding capacity

WHC of all batches was very high and fluctuated between 77-88% throughout the storage period (Table 2). After 8 days of freezing, only the shrimp treated with formulations that included gluconic acid or glucose showed higher (but not significant) WHC values than that of the control sample. From then on, and up to the end of the storage, the WHC remained stable or presented a slight increase in almost all lots. That is, the treatments tested did not cause any alteration in the water binding properties of shrimp muscle protein during storage. This fact is very important and demonstrates that the melanosis-inhibiting formulations used in this work did not produce undesirable changes in the functional quality of myofibrillar proteins during the freezing/thawing processes. As well, the high WHC of shrimp muscle proteins indicated a good functionality even though cryoprotectants were not included in the formulations used.

3.2. Shear strength

The treatments with 4-HR-based formulations produced an increase of shear resistance, which was more pronounced in the lot treated with R-0.25 (Fig. 1a). Nonetheless, the increment of shear strength was not dependent on the 4-HR concentration in the blends and was not always significant. Moreover, shrimps treated with sulphite-based formulations showed a significant hardening of muscle at the beginning of frozen preservation, compared to the control batch without additives (Figure 1b). The only exception was the CS-GLU lot.

During frozen storage, the shear strength of muscle shrimps remained stable or slightly increased. It is worth mentioning that, in general, the incorporation of either chitosan, gluconic acid or glucose counteracted the hardening effect of the sulphite-based formulation. The increment of hardness observed in all samples, mainly at the end of frozen storage, was also observed by Schubring (2002) in frozen brown shrimp. The reason could be the production of formaldehyde which may lead to myofibrillar protein aggregation. It will negatively affect texture parameters.

3.3. pH

Initially, the pH of newly caught shrimp was 7.3±0.1. Values in this range have been obtained by Bono, Badalucco, Cusumano, & Palmegiano (2012) and López-Caballero et al. (2019) for this crustacean species. The pH of all lots was similar at the beginning of the storage (7.35-7.46, data not shown), and increased slightly throughout 6 months of frozen storage, reaching values at the end of 7.37-7.56. In general terms, shrimps treated with melanosis inhibiting blends showed values slightly higher than those of the control

sample, but the pH values were always below 8, considered as a limit of acceptability for this product. In this regard, the use of organic acids in the formulations did not decrease the pH in the muscle when compared with the control sample. This fact could be attributed to the buffering capacity of the shrimp muscle. Similar pH values (around 7.4-7.5) were also reported by Condurso, et al. (2016) at the beginning and at the end of frozen storage of rose shrimp. The slight increase in pH after frozen storage has been also observed by Bono et al. (2012), and could be due to the production of dimethylamine (DMA).

3.4. Microbial counts

The microbiological counts of all samples are shown in Table 3. Upon arrival at the laboratory, the frozen shrimp registered total bacterial counts from 2.3 log cfu/g (batch CS-GLN) to 4.3 log cfu/g (batch R-0.25), ($p \leq 0.05$). In general, total flora counts were close to those obtained by various authors even in freshly caught shrimp (Mendes, Huidobro, & Caballero, 2002; López-Caballero, Gonçalves, & Nunes, 2002). Batches treated with blends R-0.1 and R-0.05 registered counts below the detection limit. H_2S-producer microorganisms were only detected in batches treated with 4-HR-based formulations (0.05 and 0.25%), whereas in the rest of the batches they remained within values below the limit of detection ($p \leq 0.05$). The genus Pseudomonas, implicated in refrigerated seafood spoilage (López-Caballero et al., 2019), was detected in 4 of the 9 batches under study, although in a very low number ($p < 0.05$). Lactic acid bacteria were found in all batches at the beginning of storage (Table 3), in concentrations ranging from 1 log cfu/g in shrimp treated with R-0.1 and with CS and up to 2.2 cfu/g in the CS-GLN lot. This fact would indicate that the addition of gluconic acid to sulphites could favour the development of this group of bacteria capable of growing at low pH. Enterobacteria were detected at a low concentration ($p \leq 0.05$). Some authors have explained that the application of formulations containing 4-HR (López-Caballero, Martínez-Alvarez, Gómez-Guillén, & Montero, 2007) or chitosan (López-Caballero, Martínez-Álvarez, Gómez-Guillén, & Montero, 2006a; Yuan, Zhang, Tang, & Sun, 2016) on crustaceans reduces the microbiota in comparison with the application of formulations with conventional sulphites. In the present work this possibility could be masked by the rapid decrease in temperature after the application of the treatments.

In most of the lots, a decrease in the number of microorganisms was observed due to frozen storage ($p \leq 0.05$). Freezing is not a sterilization process and thus cannot destroy microorganisms, but may cause stress due in part to the decrease in water availability,

which is necessary for their multiplication and results in death or viability loss of part of the microbial population. Fatima, Khan, & Qadri (1988) obtained a logarithmic unit decrease in the total aerobic plate counts by partially freezing shrimp (*Penaeus merguiensis*) at -3° C. The counts of H2S-producer microorganisms, Pseudomonas and *Enterobacteriaceae* descended significantly after 15 days of frozen storage and were not even detected at the end of the study period. As for the total flora, they decreased approximately in 1 logarithmic cycle after 15 days of freezing. After 6 months of storage, the total bacteria count in the lots treated with commercial sulphites (alone or in combination) and in the CR lot were close to the limit of detection. With a large sample size (1300 units from commercial grocers, different brands), Swartzentruber, Schwab, Duran, Wentz, & Read (1980) reported that the majority of samples of raw frozen in-shell shrimp reached an approximate range of aerobic plate count of 10^6 value/g. The conservation time of the shrimp as well as their treatments were not specified; however, since they were commercial samples (within their shelf life), they supposedly showed standard values. Similarly, Singh, Chan, Ng, & Yong (1987) reported that 76% of frozen seawater shell-on shrimp (for import or export samples) were of good quality ($\leq 10^6$ cfu/g) according to recommendations (ICMSF, 1986). Lactic acid bacteria were present during almost all of the storage period, except in the R-0.1 batch ($p < 0.05$). On the contrary, López-Caballero et al. (2006b) reported that 4-HR-based formulations (0.1%) appeared to inhibit the growth of lactic acid bacteria during Norway lobster chilled storage.

3.5. Volatile compounds

Shrimp presented TVB-N values of 22.5 mg/100 g prior to freezing (Table 4). In most cases, the formulations increased the concentration of TVB-N compounds ($p \leq 0.05$), reaching 27.9 mg/100 g in the lot R-0.1. The lot treated with sulphites registered 25.9 TVB-N mg/100 g. The rest of the formulations, 4-HR-based or sulphite-based, recorded intermediate values. Bono et al. (2012) reported that in deep-water rose shrimp treated with sulphites, the TVB-N values increased (42 mg/100 g) compared with those of shrimp vacuum packed or modified atmosphere packed (33.5 or 36.5 mg/100 g, respectively). These authors attributed the increase in volatile compound concentration, especially in shrimp treated with sulphites, to the formation of DMA from trimethylamine oxide (TMAO) by non-enzymatic reactions. In the present work, determining the amount of DMA alone was considered interesting, since TMA production is assumed to be very low, as freezing limits the number of microorganisms

involved in its formation. In this respect, ammonia production by tissue enzymes far exceeds that by bacteria in newly caught deep-water rose shrimp (Martínez-Alvarez, López-Caballero, Gómez-Guillén, & Montero, 2009). Shrimp without additives presented values of 0.44 mg DMA/100 g muscle after two days of freezing. Incorporating the formulations modified slightly ($p \leq 0.05$) the concentration of this amine, with values ranging from 0.47 mg/100 g in the lot R-01 to 0.31 mg/100 g in CS-GLU ($p \leq 0.05$).

The DMA values increased in all of the batches due to the freezing effect, except in the batch without additives, which hardly showed any changes. Although the concentration reached was low in general, the largest increase with respect to the initial value corresponded to the CS, CR or HR-0.1 lots (Fig. 2). In this regard, the immersion of white shrimp (*Penaeus schmitti*) in sodium metabisulphite (in excess, 2%) for 10 minutes favoured the production of DMA and FA during frozen storage (Cintra, Ogawa, Souza, Diniz, & Ogawa, 1999). Moreover, those authors determined that the DMA content increased (to 0.65 mg/100 g) after 48 hours of frozen storage, a value which was two-fold higher after 50 days under the same storage conditions. On the other hand, several ionic constituents such as Fe^{2+}, Sn^{2+}, and $S0^{2}$ can induce the degradation of trimethylamine oxide (TMAO) to DMA, and metal chelators such as EDTA and phytic acid in the presence of Fe2+ and Sn2+ can rapidly accelerate the nonenzymatic formation of DMA in heat-dried and freeze-dried fish muscle (Spinelli & Koury, 1979). In contrast, the presence of metal chelators in some mixtures seemed not to inhibit the enzymatic formation of DMA by trimethylamine oxide demethylase. The mentioned results could argue the higher production of DMA in the aforementioned batches, an effect which is not repeated in the rest of the mixtures tested.

3.6. Colour parameters

The evolution of lightness (L*) of shrimp carapaces during frozen storage is shown in Table 5. Lightness of lots treated with sulphites was significantly higher ($p \leq 0.05$) than that of control shrimp at the beginning of frozen storage. This could be ascribed to the ability of sulphites to bleach samples, as reported by Rotllant, et al. (2002), together with the slight presence of black spots beneath the cuticles in the control sample. Lightness was also significantly higher in some lots treated with 4-HR-based formulations (R-0.25 and R-0.05). Throughout storage, fluctuations were observed in all of the batches, although there was a slight overall significant trend ($p \leq 0.05$) to increase L* values after 2.5 months of conservation, as was also observed by Condurso, et al.

(2016) in frozen narwal shrimp, and in deep-water rose shrimp. Lots R-0.25, CS and CS-GLN maintained the highest luminosity at the end of the storage period, while the control and CR samples showed the lowest. The other treatments did not show significant differences at later stages, although they led to L* values significantly higher than those of the control and CR samples ($p \leq 0.05$). These results reflect the non-appearance of melanosis in cuticles during frozen storage.

Parameter a* also offered significant differences among batches at the beginning of the storage period (Table 5). The batches treated with the highest concentrations of 4-HR (R-0.25 and R-0.1) originally showed a red coloration significantly higher ($p \leq 0.05$) than the others. These batches were followed by those treated with a lower concentration of 4-HR (CR and R-0.05) and by the one treated with sulphites and gluconic acid (CS-GLN). The reddish hue of the latest was significantly higher ($p \leq 0.05$) than that in shrimp treated with sulphites and in the control sample. During storage, a* showed a clear tendency to decrease significantly ($p \leq 0.05$) in most of the batches studied, very sharply in the case of the control sample. This fact may be due to an increase in a number of carotenoid degradation products, as observed by (Condurso, et al., 2016). The decrease in the values of a*, reaching values close to 0 and even negative, was mainly found in shrimp treated with sulphite-based formulations.

Regarding yellowness (b*), the use of sulphites appears to decrease slightly b* values at the beginning of conservation compared to the untreated batch (Table 5), except in the case of the batch treated with sulphites and chitosan (CS-CHIT). Similar results were observed in shrimp treated with 4-HR-based formulations, except for the CR lot. During the period of conservation, the yellowish colour remained practically constant or showed a slight increase in the batches studied. Regarding the sulphite treatments, the yellowness in lots CS and CS-GLN was lower than in the rest throughout conservation. In the case of the lots treated with 4-HR, R-0.25 generally registered the lowest values of yellowness throughout the storage period.

3.7. Diphenoloxidase activity

Freezing temperatures, together with the use of melanosis-inhibiting blends after capture, were not able to inhibit enzymes involved in diphenoloxidase activity completely (Fig. 3). This indicates that, although melanosis was not developed during storage, it might have appeared in the lots after thawing. Clearly, the enzymatic activity of the control sample was the highest throughout the storage. During the first month, the diphenoloxidase activity in the non-treated samples increased significantly over 30

days, and from then on it decreased up to the end of the storage. Some proPPO could be released from hemocytes, the digestive gland and chromatophores when shrimp were thawed to obtain the crude extract (Díaz-Tenorio, García-Carreño, & Pacheco-Aguilar, 2007). This proPPO could be activated by endogenous proteases, as well as inactive hemocyanin, during thawing. From the third month on, diphenoloxidase activity decreased, probably because of prolonged preservation at freezing temperatures could produce an irreversible aggregation of diphenoloxidases that decreased the oxidising activity of these enzymes.

Shrimp treated with CR also showed a high diphenoloxidase activity throughout the storage (Fig. 3b). In contrast, shrimp treated with 4-HR-based formulations, including acids and protease inhibitors (R-0.05, R-0.1, R-0.25), showed the lowest values of diphenoloxidase activity (Fig, 3a). The diphenoloxidase inhibiting activity of these blends has been previously documented in refrigerated crustaceans (López-Caballero et al., 2019). The diphenoloxidase-inhibiting effect of R-0.25 was the highest in some phases of the storage, but it cannot be concluded that this effect depended on the concentration of 4-HR used. Moreover, the significant differences between the effect of CR and that of R-0.05, R-0.1 and R-0.25 cannot be attributed to the concentration of 4-HR used (0.8% of commercial product), but to the time of exposure to the treatment recommended by the manufacturer (five-minute immersion). This period seems to be insufficient to allow adequate diffusion of 4-HR in deep-water rose shrimp meat.

The incorporation of glucose in the commercial sulphite-based formulation (CS-GLU) did not improve the effect of commercial sulphites (CS), unlike the incorporation of gluconic acid (Fig. 3b), suggesting that this chemical may have a synergic effect with sulphites against melanosis, as reported by López-Caballero, et al. (2006a).

3.8. Melanosis score

Fig 4 and 5 shows the evolution of melanosis in treated shrimp during frozen storage. Once the crustaceans were thawed, the score was immediately carried out. Initially, slight blackening was perceived in some lots, which is attributed to the time passed between capture and freezing. Deep-water rose shrimp is very susceptible to melanosis, being able to present a considerable level of black spots within the first 48 hours after capture (López-Caballero et al., 2019).

The lots with significant higher initial melanosis index were the control lot (NA), and the lots treated with sulphites, except lot CS-GLN (Fig. 4b). In contrast, all the batches treated with 4-HR-based formulations offered a total absence of black spots at the

beginning of frozen storage (Fig. 4a). In the first month of storage, a slight but significant increase ($p \leq 0.05$) in the melanosis level was observed in the control lot and in several lots treated with sulphites (CS-CHIT and CS-GLU) or with commercial 4-HR-based formulation (CR). The first days of freezing constitute a critical period in fishery products, since usually reorganization and conformational changes in muscle constituents take place, especially in proteins, as a consequence of the water crystallization phenomenon due to low temperatures. The enzymatic mechanism of polyphenol oxidase (PPO), as well as the subsequent polymerization of quinones, may have been affected as part of these processes, especially in the control lot and in the most ineffective treated batches, mainly CS-CHIT and CS-GLU. These batches registered a melanosis index throughout the storage period equal to or greater than 2, which could compromise the marketability of these shrimp. Chitosan has been reported as an effective antimicrobial agent and a possible melanosis-inhibiting agent at concentrations equal to or greater than 2% (Simpson, et al., 1997), while at a lower concentration it shows a very limited effectiveness in the prevention of blackening, despite the presence of sulphites in the formulation. Nonetheless, these authors noticed that both the degree of acetylation and the molecular weight of the chitosan used have influence on the antimicrobial efficacy. Similarly, the formula CS-GLU was ineffective to prevent the appearance of black spots throughout the storage period. The sulphite batch showed a significant tendency ($p \leq 0.05$) to present black spots within the first month and a half of freezing. However, this tendency was surprisingly reversed in the following months, to reach practically imperceptible levels of melanosis at the end of the conservation period. Apart from a possible heterogeneity in the distribution of the blend, these changes could be related to variations in the interaction of sulphites with phenols and quinones at different stages of oxidation and polymerization. The presence of gluconic acid in the sulphite formulation (CS-GLN) effectively prevented the appearance of melanosis from the beginning of the shelf life, remaining unchanged until the sixth month of storage. About shrimp treated with 4-HR-based formulations, almost all treatments were effective in preventing the appearance of melanosis, with no significant differences ($p \leq 0.05$) between the concentrations tested.

3.9. Odour

Table 6 represents the evolution of shrimp odour throughout frozen storage. Although differences were very subtle, immediately after freezing, the shrimp treated with 4-HR showed a fresher odour when compared with those treated with sulphites. This slight

difference can be attributed to a better protection of 4-HR against autolytic and microbial degradation in the hours before freezing as compared to that caused by sulphites. In any case, after two months of conservation, all the batches registered scores around 1 (neutral odour), including the control lot, remaining relatively constant throughout the subsequent period of storage.

4. Conclusions

Shrimp treated with different melanosis-inhibiting blends maintained good sensory, functional and microbial quality during six months of frozen storage at -18ºC. The treatments applied produced some differences in the analysed parameters compared with the control sample, mainly at the beginning of the storage in the frozen state. These differences were noticeable in relation to the melanosis index and were attributed to the time elapsed from shrimp capture to the moment of freezing, where enzymatic deterioration mechanisms, including PPO activity and all autolytic phenomena, are more relevant than microbial deterioration. The treatments with 4-HR-based formulations, regardless of the concentration tested, were very effective in preventing melanosis before freezing and subsequently throughout frozen storage. The conventional treatment with EverFresh® was also effective in avoiding the appearance of black spots prior to freezing. Moreover, the joint use of sulphites and gluconic acid ensured the practical absence of melanosis throughout the conservation period, without any alteration in the rest of the properties studied. In conclusion, the inhibiting blends were useful to preserve good sensorial, functional and microbial quality in shrimp after six months of frozen storage.

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635

Table 1: Melanosis-inhibiting blends applied by immersion or spraying on deepwater pink shrimp after capture. The concentration of solid chemicals is specified in % per weight of shrimp. The concentration of liquid chemicals is specified per volume of water (N). The commercial inhibiting-blend Freskor (CS) included sodium metabisulphite (60% maximum content), citric acid, ascorbic acid, EDTA and sodium bicarbonate (unknown concentrations).

BLEND	4-Hexyl-resorcinol	Citric acid	Ascorbic acid	Acetic acid	EDTA	PPi	Sodium metabisulphite	Gluconic acid	Glucose	Chitosan	Sodium Chloride
NA											
R 0.25	0.25%	0.50%	0.50%	0.05N	450 ppm	1.50%					
R 0.1	0.10%	0.50%	0.50%	0.05N	450 ppm	1.50%					
R 0.05	0.05%	0.50%	0.50%	0.05N	450 ppm	1.50%					
CS		unknown	unknown		unknown		1.1-1.2%				
CS-GLN		unknown	unknown		unknown		1.1-1.2%	0.15N			
CS-CHIT		unknown	unknown	0.05N	unknown		1.1-1.2%			0.30%	
CS-GLU		unknown	unknown		unknown		1.1-1.2%		6%		
CR	unknown										unknown

Table 2: Water holding capacity of treated shrimps during frozen storage. The results were expressed as percentage of total water retained (mean ± SD). Different letters (a,b,c,…) in the same row indicate significant differences among samples treated with the same blend at different days of preservation. Different letters (x,y,z…) in the same column indicate significant differences among lots at the same day of storage.

	Days of storage					
	8	20	40	96	140	180
R-0.25	$77.8\pm0.3^{a/xy}$	$80.3\pm2.5^{ab/x}$	$78.5\pm1.9^{a/x}$	$81.6\pm1.3^{a/x}$	$82.5\pm0.8^{a/x}$	$82.6\pm3.0^{a/x}$
R-0.1	$77.2\pm1.6^{a/xy}$	$79.7\pm2.4^{a/x}$	$78.4\pm2.0^{a/x}$	$82.6\pm1.0^{a/x}$	$82.8\pm0.6^{a/x}$	$82.8\pm0.3^{a/x}$
R-0.05	$80.0\pm1.4^{a/xy}$	$79.6\pm2.0^{a/x}$	$82.9\pm0.9^{a/x}$	$82.4\pm0.2^{a/x}$	$80.4\pm1.5^{a/x}$	$82.1\pm0.9^{a/x}$
CS	$79.0\pm1.7^{a/xy}$	$79.7\pm2.0^{ab/x}$	$84.1\pm3.4^{ab/x}$	$84.1\pm1.7^{b/x}$	$85.3\pm1.7^{b/x}$	$84.1\pm1.5^{b/x}$
CS-GLN	$82.5\pm1.0^{ab/y}$	$77.1\pm6.3^{a/x}$	$81.1\pm1.2^{ab/x}$	$83.1\pm0.8^{ab/x}$	$83.6\pm1.8^{b/x}$	$80.6\pm4.4^{ab/x}$
CS-CHI	$79.8\pm0.7^{a/xy}$	$81.6\pm2.9^{a/x}$	$83.8\pm1.0^{a/x}$	$83.3\pm0.1^{a/x}$	$83.6\pm1.5^{a/x}$	$84.3\pm1.9^{a/x}$
CS-GLU	$82.0\pm1.3^{ab/y}$	$80.0\pm2.4^{a/x}$	$81.4\pm1.4^{ab/x}$	$82.9\pm1.7^{ab/x}$	$83.0\pm1.6^{ab/x}$	$86.8\pm1.3^{b/x}$
CR	$74.0\pm0.7^{a/x}$	$84.6\pm3.2^{b/x}$	$81.3\pm3.6^{b/x}$	$82.3\pm1.8^{b/x}$	$83.2\pm2.9^{b/x}$	$80.7\pm2.0^{b/x}$
NA	$80.8\pm1.1^{a/xy}$	$80.0\pm1.7^{a/x}$	$77.7\pm4.5^{a/x}$	$81.0\pm2.3^{a/x}$	$82.8\pm1.4^{a/x}$	$84.0\pm2.6^{a/x}$

Table 3. Microbial counts in deepwater pink shrimp treated with different melanosis-inhibiting formula during frozen storage. Results were expressed as log cfu/g. Detection limits: <2 log cfu/g for total bacteria count, H_2S-producing microorganisms, luminescent colonies and *Pseudomonas* spp; <1 log cfu/g for lactic acid bacteria and Enterobacteriaceae. Different letters (a, b, c...) in the same raw indicate significant differences ($p \leq 0.05$) as a function of treatment; different letters (x, y, z...) in the same column indicate significant differences ($p \leq 0.05$) as a function of storage time.

		Blend								
Microorganisms	**Storage (months)**	**R-0.25**	**R-0.1**	**R-0.05**	**CS**	**CS-GLN**	**CS-CHIT**	**CS-GLU**	**CR**	**NA**
Total bacteria counts	0	$4.3^{b/-}$	<2	<2	$3.2^{c/y}$	$3.0^{c/y}$	$3.0^{c/z}$	$2.3^{d/x}$	$2.1^{d/y}$	$3.1^{c/z}$
	0.5	<2	<2	<2	$2.2^{b/x}$	$2.4^{b/x}$	$2.2^{b/y}$	$2^{b/x}$	<2	$2.1^{b/y}$
	6	<2	<2	<2	$2.1^{b/x}$	$2.6^{c/xy}$	<2	$2.1^{b/x}$	$2.8^{c/z}$	<2
H_2S producer organisms	0	$2.3^{b/-}$	<2	$2.0^{b/-}$	$2^{b/-}$	<2	<2	<2	<2	<2
	0.5	<2	<2	<2	<2	2.0	<2	<2	<2	<2
	6	<2	<2	<2	<2	<2	<2	<2	<2	<2
***Pseudomonas* spp.**	0	<2	$2.0^{b/-}$	<2	<2	$2.1^{b/-}$	$2.3^{b/y}$	<2	<2	$2.1^{b/y}$
	0.5	<2	<2	<2	<2	<2	$2.3^{b/y}$	$2^{b/-}$	<2	<2
	6	<2	<2	<2	<2	<2	<2	<2	2.0	<2
LAB	0	$1.6^{b/y}$	$1.0^{a/-}$	$1.2^{a/y}$	$2.1^{c/x}$	$2.2^{c/y}$	$1.7^{b/x}$	$1.5^{ab/x}$	$1.0^{a/x}$	$2.1^{c/x}$
	0.5	$1.1^{b/x}$	<1	<1	$1.9^{c/x}$	$1.1^{b/x}$	$1.3^{b/x}$	$1.2^{b/x}$	$1.4^{b/x}$	$1.6^{c/x}$
	6	$1.1^{b/x}$	<1	$1.3^{b/y}$	$1.6^{c/x}$	$2.0^{c/y}$	$1.3^{b/x}$	$1.3^{b/x}$	$2.5^{d/y}$	$1.9^{cd/x}$
Enterobacteriaceae	0	$1.9^{b/-}$	$1.3^{a/-}$	$2.3^{b/-}$	$1.5^{a/-}$	$1.6^{ab/-}$	$1.3^{a/y}$	$1.0^{a/-}$	$1.3^{a/y}$	$1.3^{a/-}$
	0.5	<1	<1	<1	<1	<1	$1.0^{-/y}$	<1	<1	<1
	6	<1	<1	<1	<1	<1	<1	<1	$1.2^{-/y}$	<1

Table 4.- Total volatile basic nitrogen content in shrimp before freezing. Results were expressed as mg NBVT/100 g of muscle (mean ± SD). Different letters (x, y, z...) in the same column indicate significant differences ($p<0.05$) as a function of storage time.

Blend	**mg TVB-N**
R-0.25	25.47 ± 0.50^{abc}
R-0.1	27.96 ± 1.19^{a}
R-0.05	23.19 ± 1.89^{bcd}
CR	21.86 ± 0.98^{bc}
CS-GLN	24.15 ± 0.28^{abcd}
CS-CHIT	22.43 ± 1.03^{bcd}
CS-GLU	21.29 ± 0.06^{b}
CS	25.99 ± 0.38^{ad}
NA	22.51 ± 1.21^{bcd}

Table 5: L*, a* and b* values (mean ± SD) of unpeeled shrimps during frozen storage. Different letters **(**a,b,c,…) indicate significant differences among samples treated with the same blend at different days of preservation. Different letters (x,y,z…) indicate significant differences among lots at the same day of storage.

		Days of storage					
Blend		**0**	**15**	**50**	**90**	**140**	**180**
R 0.1	**L***	51.22 ± 2.11$^{a/u}$	62.12 ± 0.78$^{b/vx}$	61.44 ± 0.75$^{b/y}$	67.28 ± 0.98$^{c/x}$	68.84 ± 0.70$^{c/vx}$	68.83 ± 1.25$^{c/v}$
	a*	2.82 ± 0.88$^{b/xy}$	2.82 ± 0.71$^{b/v}$	4.11 ± 1.49$^{b/y}$	0.69 ± 0.72$^{a/vx}$	0.59 ± 0.71$^{a/v}$	1.35 ± 0.55$^{a/vx}$
	b*	14.16 ± 1.73$^{a/x}$	15.06 ± 1.37$^{ab/xy}$	15.91 ± 1.56$^{bc/x}$	17.09 ± 1.83$^{c/x}$	17.35 ± 0.62$^{c/xy}$	17.75 ± 0.86$^{c/vxy}$
R 0.05	**L***	64.21 ± 1.19$^{b/yz}$	65.10 ± 0.92$^{b/xy}$	57.03 ± 1.66$^{a/x}$	67.44 ± 0.83$^{b/x}$	65.84 ± 0.69$^{b/v}$	67.58 ± 0.86$^{b/v}$
	a*	1.55 ± 0.67$^{b/v}$	2.99 ± 0.90$^{c/v}$	3.15 ± 0.86$^{c/y}$	2.51 ± 1.91$^{bc/x}$	0.10 ± 0.83$^{a/v}$	3.11 ± 0.68$^{b/x}$
	b*	12.54 ± 1.77$^{a/u}$	15.20 ± 1.04$^{bc/y}$	15.21 ± 1.65$^{b/x}$	16.79 ± 1.37$^{cd/x}$	19.05 ± 0.73$^{e/z}$	13.45 ± 0.75$^{de/xy}$
R 0.25	**L***	65.77 ± 1.51$^{b/yz}$	67.69 ± 0.74$^{b/yz}$	61.09 ± 0.90$^{a/y}$	68.41 ± 0.71$^{b/x}$	67.72 ± 1.97$^{b/vx}$	73.14 ± 0.44$^{b/v}$
	a*	3.77 ± 1.31$^{cd/y}$	5.00 ± 0.99d$^{/x}$	1.56 ± 1.45$^{ab/vx}$	1.74 ± 1.06$^{b/xy}$	0.48 ± 0.83$^{a/v}$	1.33 ± 0.69$^{c/y}$
	b*	12.65 ± 1.03$^{ab/vx}$	12.96 ± 0.70$^{ab/u}$	12.82 ± 0.92$^{ab/u}$	13.43 ± 1.33$^{b/uv}$	11.58 ± 0.93$^{a/u}$	13.71 ± 1.02$^{b/u}$
CS	**L***	61.65 ± 1.78$^{a/xy}$	69.56 ± 0.55$^{b/z}$	60.60 ± 2.38$^{a/y}$	68.07 ± 2.07$^{b/x}$	69.70 ± 1.33$^{b/x}$	73.14 ± 0.61$^{c/y}$
	a*	-0.36 ± 0.41$^{a/u}$	2.61 ± 0.53$^{d/v}$	1.67 ± 1.22$^{cd/vx}$	-0.17 ± 0.64$^{a/uv}$	0.12 ± 0.84$^{ab/v}$	1.33 ± 0.98$^{bc/x}$
	b*	12.66 ± 1.53$^{ab/vx}$	12.47 ± 1.24$^{a/u}$	12.49 ± 1.38$^{a/u}$	14.28 ± 0.95$^{b/v}$	12.25 ± 0.30$^{a/uv}$	13.71 ± 0.57$^{ab/u}$
CS-GLN	**L***	64.58 ± 2.47$^{b/yz}$	63.29 ± 1.00$^{b/x}$	59.59 ± 1.37$^{a/xy}$	71.94 ± 0.70$^{d/y}$	68.47 ± 1.63$^{c/vx}$	72.24 ± 0.66$^{d/xy}$
	a*	1.56 ± 1.11$^{cd/vx}$	2.81 ± 0.82$^{d/v}$	1.45 ± 0.72$^{c/v}$	0.47 ± 0.64$^{bc/uv}$	-0.40 ± 0.80$^{ab/v}$	-1.26 ± 1.59$^{a/u}$
	b*	13.96 ± 0.64$^{b/vx}$	13.84 ± 1.02$^{b/uxy}$	11.73 ± 0.67$^{a/u}$	12.73 ± 0.68$^{ab/u}$	13.39 ± 1.38$^{b/v}$	15.71 ± 0.96$^{c/v}$
CS-CHIT	**L***	60.15 ± 3.11$^{b/x}$	59.67 ± 1.95$^{b/v}$	53.01 ± 1.10$^{a/v}$	65.41 ± 1.29$^{c/vx}$	67.89 ± 1.01$^{cd/vx}$	69.16 ± 0.87$^{d/vx}$
	a*	-0.26 ± 0.62$^{a/u}$	-0.21 ± 1.54$^{a/u}$	2.77 ± 0.38$^{b/xy}$	-1.03 ± 0.68$^{a/u}$	-0.19 ±0.40$^{a/v}$	0.08 ± 0.55$^{a/v}$
	b*	14.60 ± 2.25$^{b/xy}$	12.48 ± 2.26$^{a/u}$	16.47 ± 1.12$^{c/x}$	17.67 ± 0.75$^{cd/x}$	18.42 ± 0.92$^{d/yz}$	17.38 ± 0.71$^{cd/vxy}$
CS-GLU	**L***	67.63 ± 1.21$^{c/z}$	59.49 ± 2.43$^{b/v}$	49.89 ± 2.72$^{a/v}$	67.46 ± 1.19$^{b/uv}$	67.48 ± 0.91$^{c/vx}$	68.02 ± 0.81$^{c/v}$
	a*	0.46 ± 0.94$^{ab/u}$	2.77 ± 1.15$^{a/v}$	3.39 ± 0.91$^{c/y}$	-0.71 ± 0.97$^{a/u}$	-0.15 ± 1.19$^{ab/v}$	0.63 ± 0.35$^{ab/vx}$
	b*	12.50 ± 0.97$^{a/uv}$	13.46 ± 1.95$^{a/ux}$	15.73 ± 0.72$^{b/x}$	17.04 ± 1.38$^{b/x}$	16.24 ± 1.20$^{b/x}$	16.79 ± 0.49$^{b/vx}$
CR	**L***	54.94 ± 1.84$^{a/uv}$	63.07 ± 1.33$^{bc/x}$	62.59 ± 0.85$^{b/y}$	64.65 ± 2.35$^{bc/vx}$	65.65 ± 0.74$^{c/v}$	61.87 ± 0.81$^{b/u}$
	a*	1.64 ± 1.03$^{b/vx}$	1.89 ± 0.91$^{b/v}$	1.12 ± 0.72$^{b/uv}$	-0.23 ± 0.70$^{a/uv}$	0.19 ± 0.49$^{ab/v}$	0.69 ± 1.05$^{ab/vx}$
	b*	17.01 ± 2.35$^{b/z}$	14.91 ± 1.26$^{ab/xy}$	16.81 ± 2.39$^{b/x}$	17.20 ± 1.07$^{b/x}$	16.43 ± 0.36$^{b/x}$	17.51 ± 1.21$^{b/xy}$
NA	**L***	55.25 ± 2.83$^{b/v}$	59.12 ± 1.93$^{cd/v}$	51.30 ± 1.85$^{a/v}$	59.28 ± 1.09$^{cd/u}$	58.46 ± 1.37$^{bc/u}$	61.90 ± 1.50$^{d/u}$
	a*	-0.23 ± 0.90$^{b/u}$	0.20 ± 1.00$^{b/u}$	0.10 ± 0.53$^{b/u}$	-0.84 ± 1.16$^{b/u}$	-2.75 ± 1.32$^{a/u}$	-1.90 ± 1.42$^{a/u}$
	b*	15.84 ± 1.12$^{b/yz}$	13.20 ± 1.55$^{a/u}$	16.30 ± 1.16$^{b/x}$	14.82 ± 1.00$^{b/v}$	16.70 ± 1.18$^{b/x}$	18.36 ± 1.21$^{c/y}$

Table 6: Odor of shrimps during frozen storage.

	Days of storage					
Blend	**0**	**15**	**60**	**90**	**150**	**180**
R-0.25	0.2±0.4	0.2±0.3	1.0±0.1	1.0±0.0	1.0±0.3	1.0±0.0
R-0.1	0.0±0.0	0.1±0.2	1.0±0.1	0.9±0.5	1.0±0.3	1.0±0.0
R-0.05	0.0±0.0	0.1±0.2	0.9±0.2	1.0±0.0	1.0±0.3	1.1±0.2
CS	0.5±0.7	0.5±0.3	1.2±0.4	1.0±0.0	0.8±0.5	1.2±0.4
CS-GLN	0.4±0.5	0.5±0.6	1.0±0.0	1.2±0.4	0.8±0.5	1.1±0.2
CS-CHI	0.2±0.4	0.5±0.3	1.2±0.4	1.2±0.4	1.1±0.4	1.1±0.2
CS-GLU	0.4±0.5	0.3±0.3	0.9±0.8	1.6±0.5	0.8±0.5	1.1±0.7
CR	0.0±0.0	0.4±0.4	1.1±0.2	1.2±0.4	0.8±0.5	0.8±0.4
NA	0.6±0.5	0.7±0.4	1.0±0.3	1.2±0.4	0.8±0.5	0.9±0.2

- Figure 1: Shear strength of shrimp muscle during frozen storage (mean ± SD).

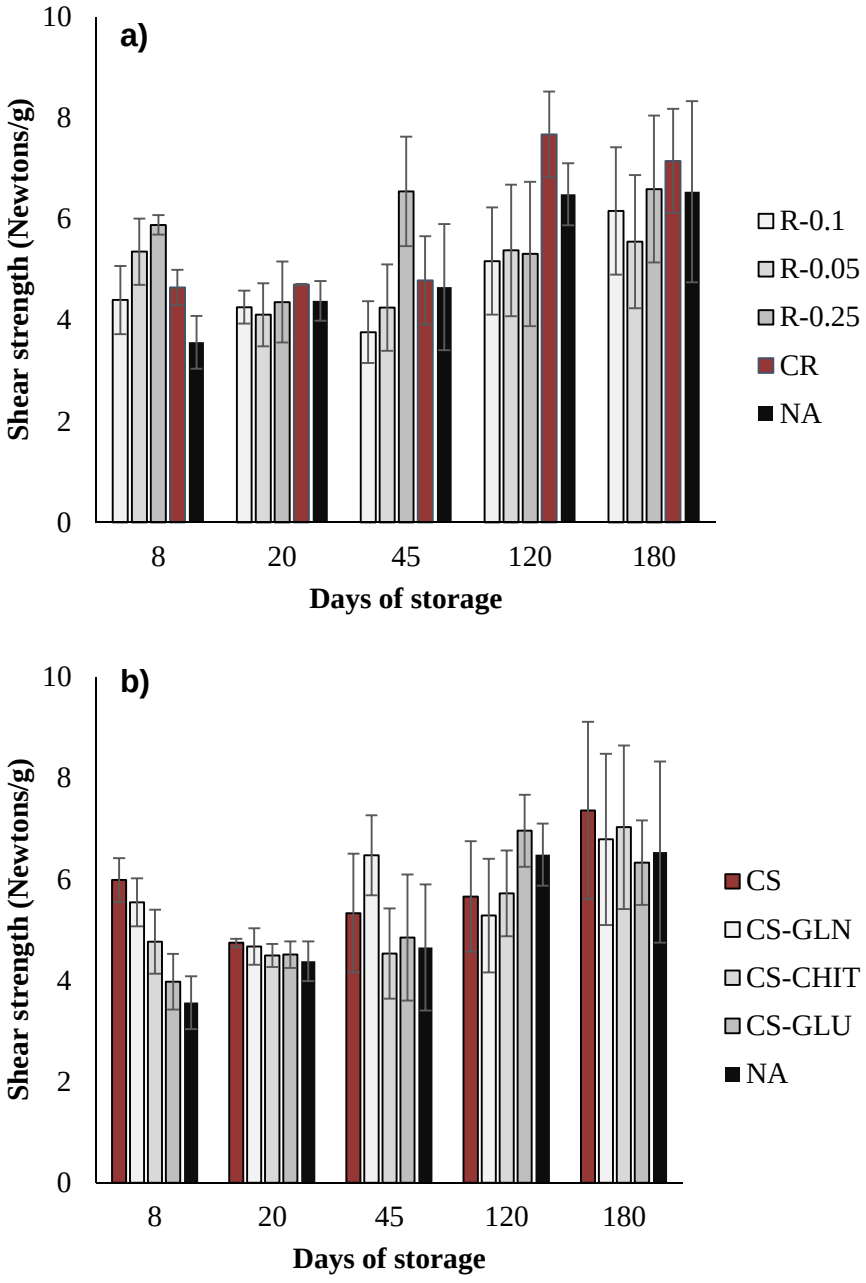
- Figure 2: DMA values (mean±SD) in shrimps before and after six months of frozen storage. The increment in DMA content was significant in all treated lots ($p<0.05$).

- Figure 3: Diphenoloxidase activity (mean±SD) of shrimps during frozen storage. * Indicates the absence of significant differences with the control sample at the same day of storage ($p<0.05$).

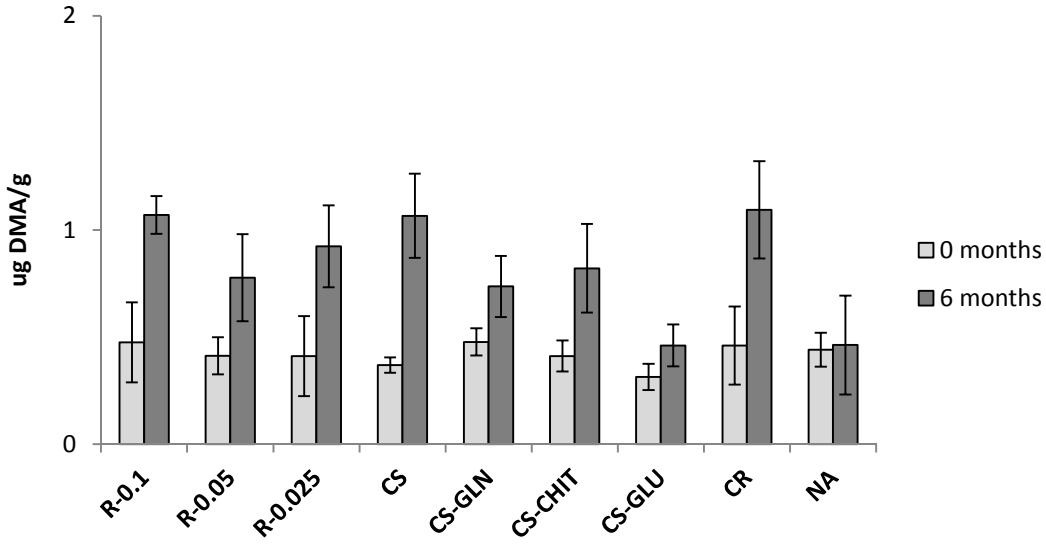
- Figure 4: Melanosis score of unpeeled shrimps during frozen storage.

- Figure 5: Appearance of shrimps before (A) and after (B) frozen storage.

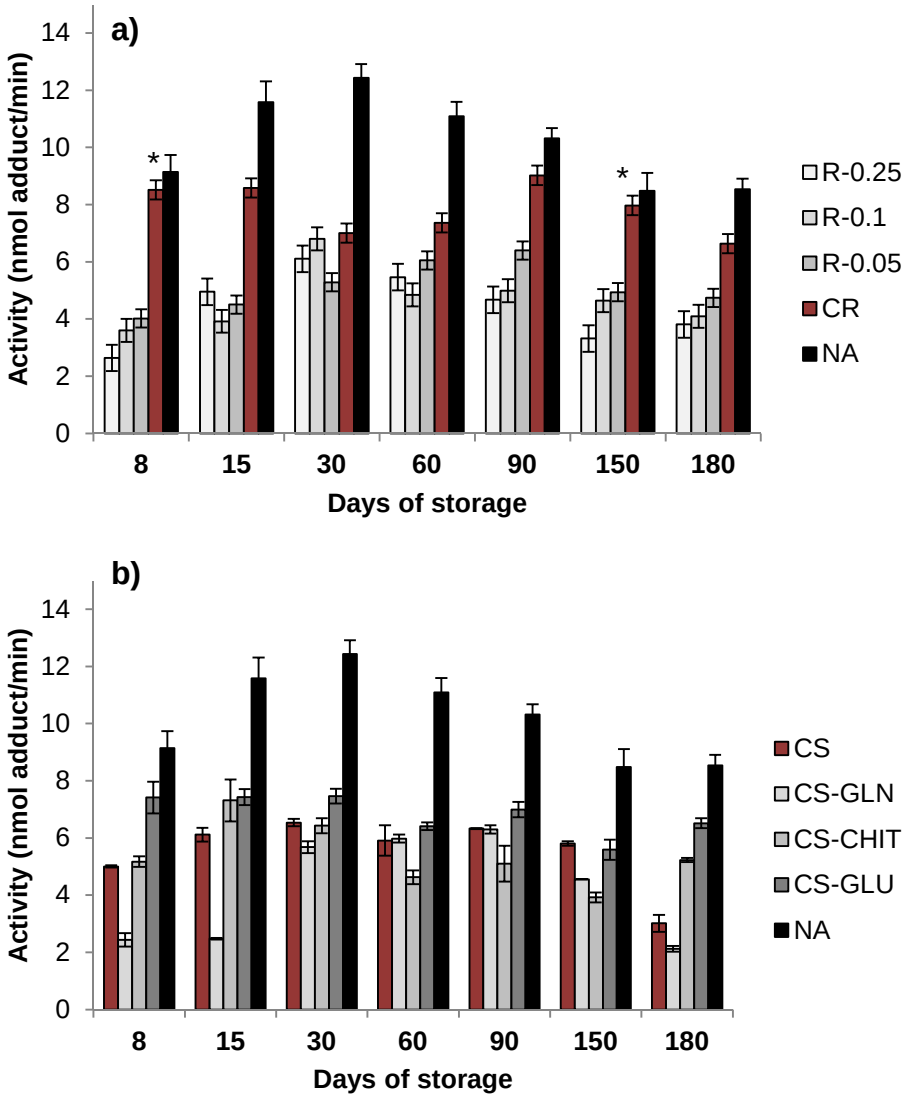
- Figure 1


a)
Shear strength (Newtons/g)
10
8
6
4
2
0
8
20
45
120
180
Days of storage
R-0.1
R-0.05
R-0.25
CR
NA
b)
Shear strength (Newtons/g)
10
8
6
4
2
0
8
20
45
120
180
Days of storage
CS
CS-GLN
CS-CHIT
CS-GLU
NA

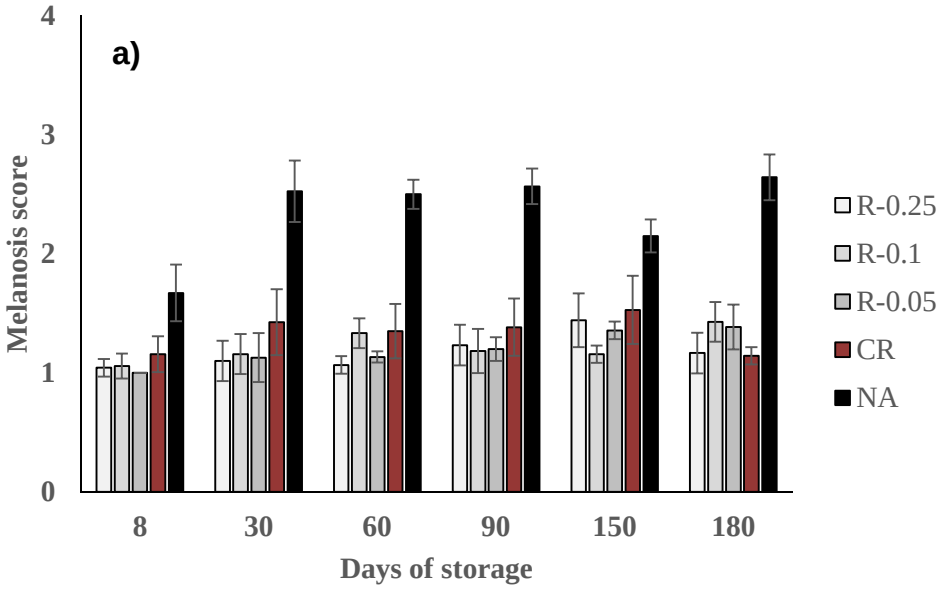
- Figure 2

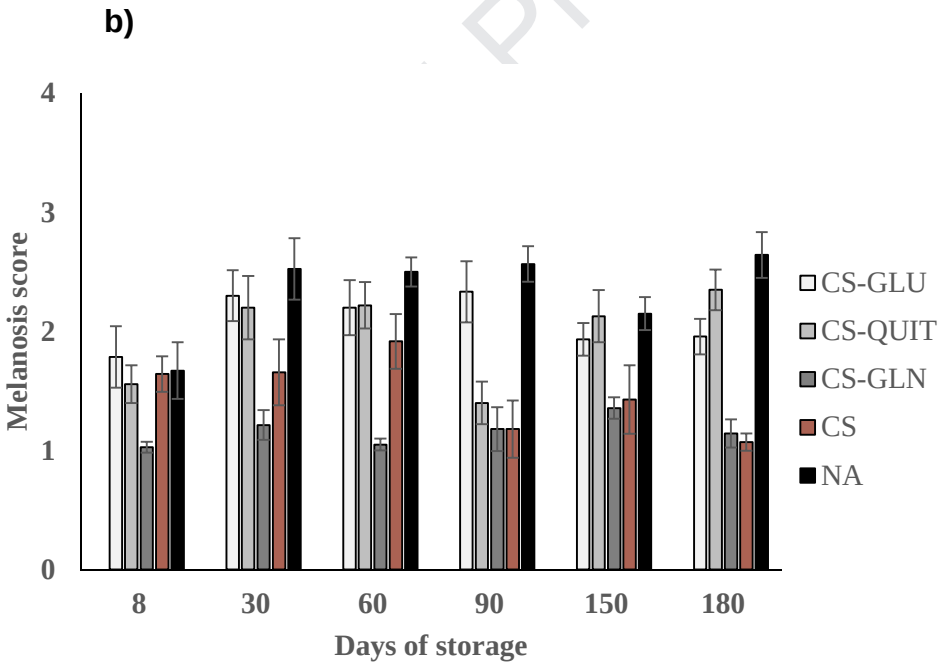


- Figure 3

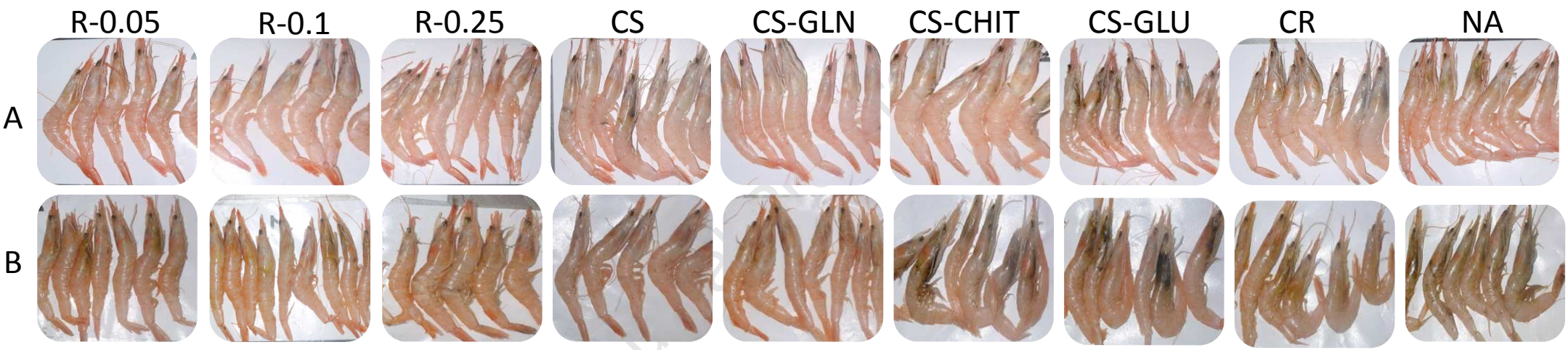

a)
Activity (nmol adduct/min)
14
12
10
8
6
4
2
0
*
*
8
15
30
60
90
150
180
Days of storage
R-0.25
R-0.1
R-0.05
CR
NA
b)
Activity (nmol adduct/min)
14
12
10
8
6
4
2
0
8
15
30
60
90
150
180
Days of storage
CS
CS-GLN
CS-CHIT
CS-GLU
NA

- Figure 4


a)
Melanosis score
0
1
2
3
4
8
30
60
90
150
180
Days of storage
R-0.25
R-0.1
R-0.05
CR
NA


b)
Melanosis score
0
1
2
3
4
8
30
60
90
150
180
Days of storage
CS-GLU
CS-QUIT
CS-GLN
CS
NA

- Figure 5



HIGHLIGHTS

- 4-Hexylresorcinol-based formula prevented melanosis in frozen shrimp
- Gluconic acid, chitosan or glucose are good coadjutants in browning-inhibiting blends
- Hardness and water holding capacity of shrimps were not affected during storage
- Formulations with 4-Hexylresorcinol reduced the total microbiota after treatment
- All formulations preserved sensory, functional and microbial quality in frozen shrimp